

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |           |  |
|---|-----------|--|
| <b>(51) International Patent Classification 5 :</b><br><b>A61K 9/127, 49/00</b>   | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 93/20800</b><br><b>(43) International Publication Date:</b> 28 October 1993 (28.10.93) |
| <b>(21) International Application Number:</b> PCT/FI93/00149<br><b>(22) International Filing Date:</b> 7 April 1993 (07.04.93)<br><br><b>(30) Priority data:</b><br>865,256                      8 April 1992 (08.04.92)                      US<br><br><b>(71)(72) Applicant and Inventor:</b> KINNUNEN, Paavo, Kai, Johannes [FI/FI]; Punarinnantie 4, SF-02660 Espoo (FI).<br><br><b>(74) Agent:</b> OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, SF-00120 Helsinki (FI).<br><br><b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). |           | <b>Published</b><br><i>With international search report.</i>   |
| <b>(54) Title:</b> COMPOSITION FOR THERAPEUTIC OR DIAGNOSTIC USE, PROCESS FOR ITS PREPARATION AND ITS USE<br><br><b>(57) Abstract</b><br><br>The present invention concerns a composition comprising a carrier selected from the group consisting of lipoproteins, in particular reconstituted LDL (Low Density Lipoprotein), other microemulsion particles, liposomes and micelles, containing a therapeutically or diagnostically active, lipo- or amphiphilic agent, and associated with a suitable ligand recognizable by its specific (complementary) cell receptor, together with a lysosomotropic agent, for targeting of the active agent to a site of interest, such as cancerous tissue.  |           |  |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                       |    |                          |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria                  | FR | France                                | MR | Mauritania               |
| AU | Australia                | GA | Gabon                                 | MW | Malawi                   |
| BB | Barbados                 | GB | United Kingdom                        | NL | Netherlands              |
| BE | Belgium                  | GN | Guinea                                | NO | Norway                   |
| BF | Burkina Faso             | GR | Greece                                | NZ | New Zealand              |
| BG | Bulgaria                 | HU | Hungary                               | PL | Poland                   |
| BJ | Benin                    | IE | Ireland                               | PT | Portugal                 |
| BR | Brazil                   | IT | Italy                                 | RO | Romania                  |
| CA | Canada                   | JP | Japan                                 | RU | Russian Federation       |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan                    |
| CG | Congo                    | KR | Republic of Korea                     | SE | Sweden                   |
| CH | Switzerland              | KZ | Kazakhstan                            | SK | Slovak Republic          |
| CI | Côte d'Ivoire            | LJ | Liechtenstein                         | SN | Senegal                  |
| CM | Cameroon                 | LK | Sri Lanka                             | SU | Soviet Union             |
| CS | Czechoslovakia           | LU | Luxembourg                            | TD | Chad                     |
| CZ | Czech Republic           | MC | Monaco                                | TG | Togo                     |
| DE | Germany                  | MG | Madagascar                            | UA | Ukraine                  |
| DK | Denmark                  | ME | Mali                                  | US | United States of America |
| ES | Spain                    | MN | Mongolia                              | VN | Viet Nam                 |
| FI | Finland                  |    |                                       |    |                          |

Composition for therapeutic or diagnostic use, process for its preparation and its use

#### FIELD OF INVENTION

5

The present invention is based on the use of a carrier selected from the group consisting of lipoproteins, in particular reconstituted LDL (Low Density Lipoprotein), other types of microemulsion particles, liposomes and micelles, and containing a therapeutically or diagnostically active, lipo- or amphiphilic agent, and associated with a suitable ligand recognizable by its specific (complementary) cell receptor, together with a lysosomotropic agent, for targeting of the active agent to the site of interest, such as cancerous tissue, and to optimize the effect thereof at the said site.

#### BACKGROUND OF THE INVENTION

20 Ligand specific receptors on the cell surface mediate both information on the environment as well as nutrients into the cell. Such ligands to be recognized include a range of molecular structures, e.g. growth factors (for example platelet derived growth factor, endothelial cell growth factor, tumor growth factor, nerve growth factor), hormones (e.g. insulin, vasopressin etc.), extracellular matrix protein (e.g. collagen) and lipoproteins (e.g. low density lipoprotein). In many cases the binding of the ligand to the cell surface receptor is followed by a clustering of the ligand-receptor complex and subsequent internalization via coated pits, ultimately locating the ligand in lysosomes. This pathway is utilized for example by viruses in their entry into cells.

35 It has been known for a long time that people suffering from cancer exhibit a cholesterol level in the serum which is lower than normal. Cholesterol circulates in the blood

in the form of a lipid-protein complex, primarily as the so-called Low Density Lipoprotein, LDL. LDL is a spherical macromolecule having a diameter of about 180 Å. LDL is surrounded by a surface film consisting of phospholipids, cholesterol and sphingomyelin, and of protein, i.e. apolipoprotein B (apo B). The interior of LDL is formed by cholesterol in esterified form (compounds of fatty acids and cholesterol), triacylglycerol, retinol, as well as other hydrocarbon soluble compounds.

Cells contain on their surfaces specific LDL-receptors which bind to the LDL-particle by specifically recognizing the protein component of the LDL-surface, i.e. apolipoprotein B. The density of LDL-receptors is much higher on cancerous cells than on normal cells and cancer cells bind LDL more effectively. It is evident that the lower serum cholesterol level of cancer patients is due to enhanced uptake of LDL by the cancer cells. This is easy to rationalize in terms of the increased requirement of components such as cholesterol for the growth of the tumor cells.

The use of reconstituted LDL containing cytotoxic compounds which replace the natural apolar lipids in the particle core has been described. The lipids are removed from the core of LDL by first lyophilizing the lipoprotein in the presence of starch (Gustafson, A (1965) J. Lipid Res. 20, 254-264). A slightly modified technique has been described by Krieger (Krieger, M. (1986) Methods Enzymol. 128, 608-613). LDL is reconstituted by lyophilizing in the presence of potato starch and extracting the neutral lipids with an organic solvent, such as heptane. The agent to be introduced is dissolved in an organic solvent and incubated with the extracted LDL. The organic solvent is evaporated and the reconstituted LDL is dissolved in a buffered aqueous solution.

According to an improvement to this method, described in the WO-publication 86/07540, the starch is replaced by a saccharide or a sugar alcohol, and according to this publication, a reconstituted LDL is obtained having similar properties as natural LDL, in vitro and in vivo.

However, when using e.g. reconstituted LDL as a carrier for e.g. drugs, in the form of an injectable aqueous solution, retro-endocytosis has been observed. Thus after an initial decrease of the drug level in the blood, indicating uptake of LDL by the cells, an increase in the level of the drug is observed, indicating expulsion of the drug from the cells. Thus it is difficult to achieve a significant accumulation of the drug in the target tissue, and consequently the action of the active agent cannot be fully taken advantage of.

It is also prior known to use, for drug delivery, liposomes wherein the active agent is incorporated a) in a core surrounded by a bilayer of a lipid substance, typically a phospholipid (together with e.g. cholesterol and sphingomyelin) or b) if the desired therapeutic compound is amphiphilic (e.g. an anti-cancer phospholipid), it is incorporated in the bilayer itself. For the said purpose also microemulsions, for example an emulsion of active agent, such as a lipophilic drug surrounded by a monolayer of phosphatidyl choline, are used.

#### SUMMARY OF THE INVENTION

The present invention relates to an improvement to the known methods by means of which it is possible to achieve an enrichment of the active agent at the target site. This is made possible by using, in combination, (a) a carrier selected from the group consisting of lipoproteins, other types of microemulsion particles, liposomes and micelles, and containing a therapeutically or a diagnostically

effective amount of a lipo- or amphiphilic active agent, associated with at least one ligand which is complementary to and recognizable by specific receptor in the living cells, and (b) a lysosomotropic agent.

5

The invention concerns, on the one hand, a composition for diagnostic or therapeutic use which contains the said combination, and on the other hand a method for the preparation of the said composition. Especially the invention concerns a composition and a method wherein the carrier is specifically reconstituted LDL, as well as its use on humans and non-human animals.

10

#### DETAILED DESCRIPTION OF THE INVENTION

15

The composition according to the invention thus contains at least two components, i.e. a carrier selected from the group of lipoproteins, other types of microemulsion particles, liposomes and micelles, and which is associated to a suitable ligand. The carrier components are known as such, as is their manner of manufacture. Common for these carriers is that they can contain a therapeutically or diagnostically active agent.

20

The carrier is associated with a ligand which is characterized in that it is complementary to and can be recognized by a specific cell receptor. The term "associated with" is intended to include also the possibility that the carrier incorporates the said ligand, such as in the case of LDL, where the ligand can be the apo B moiety included in the surface film of the LDL. The ligand is thus typically one of the substances mentioned earlier, such as a protein, e.g. growth factor, a hormone, apolipoprotein, viral protein, synthetic peptide etc. As stated, when the carrier is a reconstituted LDL, the ligand is the apo B-moiety, or a corresponding receptor recognizable synthetic peptide present on its surface. The ligand may also in all cases

30

35

be a synthetic ligand made lipophilic or amphiphilic to allow for its incorporation with the carrier, into the lipid surface of the carrier particle, provided that the ligand carries the elements recognizable by the cell receptors. Such substances are also well known in the art.

The second central component in the composition is the lysosomotropic agent which according to the invention is a lysosomotropic amphiphile, and is characterized as having both lysosomotropic and detergent activity. The lysosomotropic agents exert lysosomal blocking activity and are well known in the art. They may in addition be amphiphilic such as Triton WR-1339 (American Roland Corporation, USA, "tyloxapol"), weak amines (e.g. pK 5 to 9) such as imidazoles or morpholines containing longer hydrocarbon alkyl or acyl chains, e.g. N-dodecyl imidazole, or chloroquine, see e.g. Miller et al., J. Cell Biol. 97 (1983) 1841-1851; Wilson et al., Cancer Research 49, (1989), 507-510; and deDuve et al., Biochem. Pharmacol. 23 (1974) 2495, which are all included here for reference. When used together with the carrier and associated ligand, the lysosomotropic amphiphile due to its hydrophobic nature readily accommodates onto the carrier/particle surface. Lysosomotropic amines may in themselves have cytotoxic properties.

According to the invention it has been observed that the presence of the lysosomotropic amphiphile does not interfere with the recognition of the ligand by the receptor, but the carrier with active agent is taken up from the extracellular space into the cells according to their contents of receptors and the activity of the endocytic pathway. However, once the carrier particles carrying the lysosomotropic agent enters the lysosomes, the processing of the particles is blocked. In other words, the cycling of the active agent containing particle back to the cell surface (retro-endocytosis) does not take place, and an accumulation of the active agent in the target site is obtained.

The composition according to the invention is preferably an aqueous solution for parenteral use, such as for injection purposes (iv), containing a therapeutically or diagnostically effective amount of active agent containing carrier particles together with the lysosomotropic agent. The ratio (weight) of carrier particles to the lysosomotropic agent is suitably such that in each particle approximately 2 to 40 mole-% of its surface active compounds is constituted by the said lysosomotropic agents.

The composition according to the invention is made by combining the active agent containing carrier with the lysosomotropic agent in a pharmaceutically suitable vehicle, together with possible pharmaceutically acceptable adjuvants. The vehicle is normally sterile water. The adjuvants may be any substances known per se and suitable for the purpose, the choice thereof being within the knowledge of a person skilled in the art.

The dosage used naturally depends on the drug or diagnostic agent used, as well as the condition to be treated or diagnosed. The amount to be administered for any specific purpose can readily be determined by a person skilled in the art.

As stated above, according to a preferred embodiment, the carrier is comprised of reconstituted LDL. Such reconstituted LDL is made by a method comprising the steps of lyophilizing LDL in the presence of a protecting substance, extracting the lyophilized LDL with an organic solvent, incubating a therapeutically or diagnostically effective lipo- or amphiphilic agent in an organic solvent with the extracted LDL, evaporating the solvent and dissolving the product in an aqueous buffered solution to remove any non-included active agent and separating the LDL e.g. by ultracentrifugation.



The LDL to be reconstituted is usually isolated from human serum by differential ultracentrifugational flotation in the density range of  $1.019 < d < 1.063$  g/ml. Alternative techniques include, for instance, rate-zonal ultracentrifugation.

The protecting substance may be any of the substances known for the purpose, such as starch, in particular potato starch, but also a sugar derivative as is disclosed in the WO-publication 86/07540.

The solvent used for the extraction of the neutral lipids from the lyophilized LDL is suitably a non-polar solvent, such as heptane, hexane, pentane, petroleum ether, octane, or their mixtures.

The solvent used for dissolving the active agent and for incubating with the lyophilized and extracted LDL, is also preferably a non-polar solvent such as the ones listed above.

According to another mode of the invention, the carrier particle can be a liposome. The techniques for making liposomes and for including active agents, such as a drug therein, are very well known in the art.

In this case the ligand can comprise a suitable substance recognizable by a specific cell surface receptor, such as those listed earlier, and which is incorporated in the liposome shell during the manufacture thereof.

According to the invention also a microemulsion can be used, the preparation of which is known in the art (e.g. Walsh, M. T. et al, Methods in Enzymology, vol. 128 582 (1986). The same ligands as those mentioned above may be used.

The active agent is an agent for diagnostic or therapeutic purposes. It can be lipophilic or amphiphilic, although the border line between these two types is diffuse. As a  
5 diagnostic agent e.g. light sensitizers, such as hemato-  
porphyrins, radiosensitizers, such as boronated fatty acid  
esters, or x-ray contrast agents can be used. As a thera-  
peutic agent, preferably anti-cancer drugs are used, such  
as doxorybicin, daunomycin, 1-hexadecyl-2-methyl-3-phospho-  
10 choline etc.

The following examples illustrate the invention without  
limiting the same.

15 Example

LDL was isolated by differential ultracentrifugational  
flotation in the density range  $1.1019 < d < 1.063$  g/ml.  
Subsequently, KBr used for the adjustment of appropriate  
20 densities was removed by extensive dialysis against 0.3 mM  
EDTA, pH 7.0 at 4 °C. Thereafter the lipoprotein was  
concentrated by ultrafiltration using an Amicon XM-300  
membrane and the concentration of cholesterol was measured.  
Pure potato starch (60 mg from Sigma) was dissolved in the  
25 LDL-solution containing 16  $\mu$ moles of cholesterol in a total  
volume of 1.5 ml. The resulting solution was then frozen in  
liquid nitrogen and lyophilized. The core lipids of the  
lyophilized LDL were then extracted with 4 x 10 ml of ice-  
cold heptane. The core lipids were replaced with a cytoto-  
30 xic compound, namely cholesteryl ester of chlorambucil (15  
mg dissolved in 1.5 ml of heptane) which contained a  $^3\text{H}$ -  
cholesteryl moiety as a marker for the quantitation of  
tissue distribution of the cytotoxic compound. The solution  
was first incubated at -10 °C for 90 minutes, whereafter  
35 the solvent was removed under a gentle stream of nitrogen  
while keeping the sample on an ice-water bath. The dry  
residue was then dissolved in 3.5 ml of 10 mM Tricine

buffer, pH 8.4, and incubated at + 4 °C for 48 hours. In order to separate the cholesteryl chlorambucil loaded LDL, the solution was centrifuged at 2000 rpm and the supernatant collected. To the supernatant solution containing the  
 5 cyto-stat-LDL particles, was then added 50  $\mu$ L of Triton WR-1339, and the mixture was incubated at + 4 °C overnight. In this manner an injectable preparation was obtained. This can be used as such, or if necessary, it can be irradiated by bath type sonication to speed up the dispersion into  
 10 small particles.

#### EXPERIMENTAL

The preparation obtained in the Example above was used as  
 15 such and injected i.v. into rats bearing Rous sarcoma inoculated in their peritoneum. The average weight of the developed tumors was 0.9 g. The animals were killed after 0.5, 1.0 and 3 hrs from the injection and the radioactivities in different tissues were determined. In the following  
 20 table, test animals designated A to E are compared to the values from a control rat (killed at 1 hr) without the implanted sarcoma.

|    |          | Control | A   | B   | C   | D   | E   |
|----|----------|---------|-----|-----|-----|-----|-----|
| 25 | hrs:     | 1       | 0.5 | 1   | 1   | 3   | 3   |
|    |          | cpm/g   |     |     |     |     |     |
|    | blood    | 131     | 350 | 152 | 140 | 20  | 32  |
|    | heart    | 19      | 83  | 96  | 88  | 54  | 69  |
|    | kidney   | 22      | 44  | 56  | 72  | 56  | 85  |
| 30 | pancreas | 44      | 23  | 109 | 161 | 88  | 94  |
|    | spleen   | 47      | 33  | 120 | 153 | 213 | 151 |
|    | liver    | 85      | 79  | 189 | 195 | 299 | 187 |
|    | tumor    | -       | 23  | 459 | 530 | 296 | 471 |

35 From the results it can be seen that the LDL-chlorambucil ester accumulates in the tumor, and that the degree of expulsion is low, whereas its level in the blood decreases

rapidly to a low level.

In the appended drawing, Fig. 1 illustrates similar tests and it shows the level of chlorambucil ester-LDL in the blood and in the tumor, respectively, when injected as described above but in the absence of a lysosomotropic agent (Triton WR 1339). In Fig. 1 the abscissa indicates the time and the ordinate the amount (in promille) in blood and tumor respectively, calculated from that injected. Fig. 2 shows in graphic form the mean values for the levels obtained above, when the same LDL has been injected in the presence of the lysosomotropic agent. From the Figures it is evident that in the absence of a lysosomotropic agent, after an initial transient decrease, the level in the blood increases rapidly, whereas the opposite is true for the level in the tumour.

On the other hand, when using a preparation according to the invention, the level of reconstituted LDL in the blood decreases, and the level thereof in the tumour increases, indicating that retro-endocytosis is prevented by the incorporation of the lysosomotropic agent according to the invention.

## Claims

1. Composition for therapeutical or diagnostical use comprising, in combination, (a) a carrier selected from the group consisting of lipoproteins, other types of microemulsion particles, liposomes and micelles, and containing a therapeutically or a diagnostically effective amount of a lipo- or amphiphilic active agent, associated with at least one ligand which is complementary to and recognizable by a specific cell receptor, and (b) a lysosomotropic agent.
2. Composition according to claim 1, wherein the carrier is reconstituted Low Density Lipoprotein (LDL), the ligand being an apolipoprotein B moiety on its shell surface.
3. Composition according to claim 1 or 2, wherein the lysosomotropic agent is Triton WR 1339 ethyl oleate.
4. Composition according to claim 1 or 2, wherein the therapeutically or diagnostically active agent is selected from the group consisting of light sensitizers, radiosensitizers, x-ray contrast agents and anti-cancer drugs.
5. Composition according to claim 4, wherein the anti-cancer drug is chlorambucil cholesteryl ester.
6. Composition for therapeutical use comprising, in combination, a reconstituted Low Density Lipoprotein (LDL) containing chlorambucil cholesteryl ester and Triton WR 1339 ethyl oleate.
7. Process for the preparation of a composition for therapeutic or diagnostic use comprising the steps of combining a carrier selected from the group consisting of lipoproteins, other types of microemulsion particles, liposomes and micelles, containing a therapeutically or a diagnostically effective amount of a lipo- or amphiphilic

active agent, and associated with at least one ligand which is complementary to and recognizable by a specific cell receptor, with a lysosomotropic agent and a pharmaceutically suitable vehicle and optional further adjuvants.

5

8. Process for the preparation of a composition according to claim 2, comprising lyophilizing LDL in the presence of a protecting substance, extracting the lyophilized LDL with an organic solvent, incubating the therapeutically or  
10 diagnostically active agent in an organic solvent with the extracted LDL, removing the solvent, dissolving the reconstituted LDL in an aqueous buffered solution, and recovering the reconstituted LDL and combining a therapeutically or diagnostically effective amount of the  
15 reconstituted LDL with a lysosomotropic agent and a suitable vehicle and optionally further adjuvants.

20

9. Process according to claim 8, wherein the lysosomotropic agent is Triton WR 1339 ethyl oleate.

10. Process according to claim 8 or 9, wherein the therapeutically or diagnostically active agent is selected from the group consisting of light sensitizers, radiosensitizers, x-ray contrast agents and anti-cancer drugs.

25

11. Process according to claim 10, wherein the anti-cancer drug is chlorambucil cholesteryl ester.

30

12. Process according to claim 8, wherein the therapeutically active agent is chlorambucil cholesteryl ester and the lysosomotropic agent is Triton WR 1339 ethyl oleate.

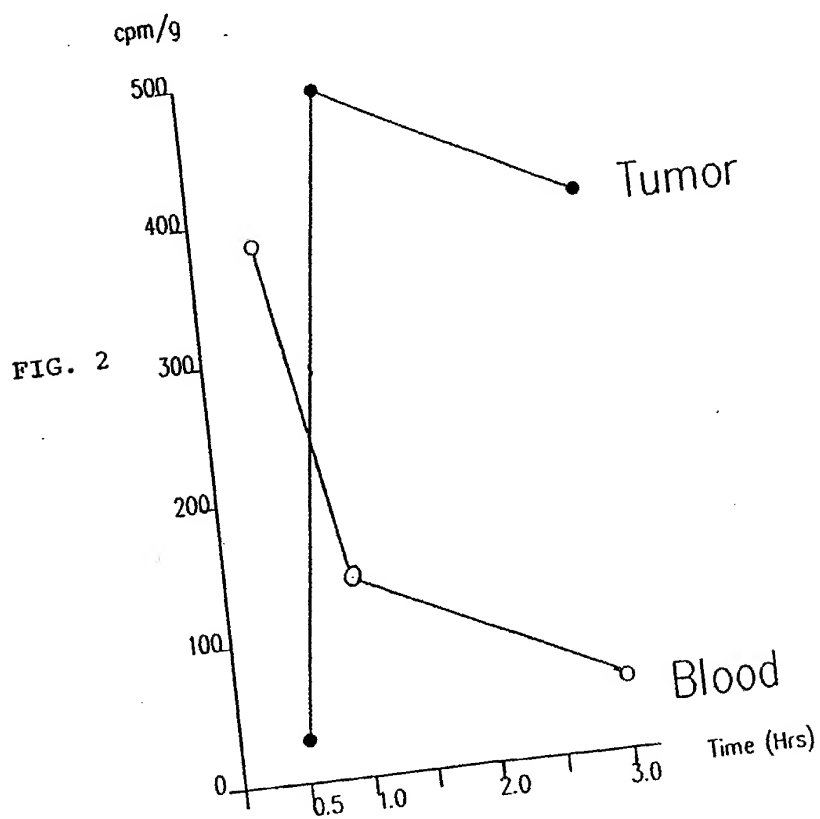
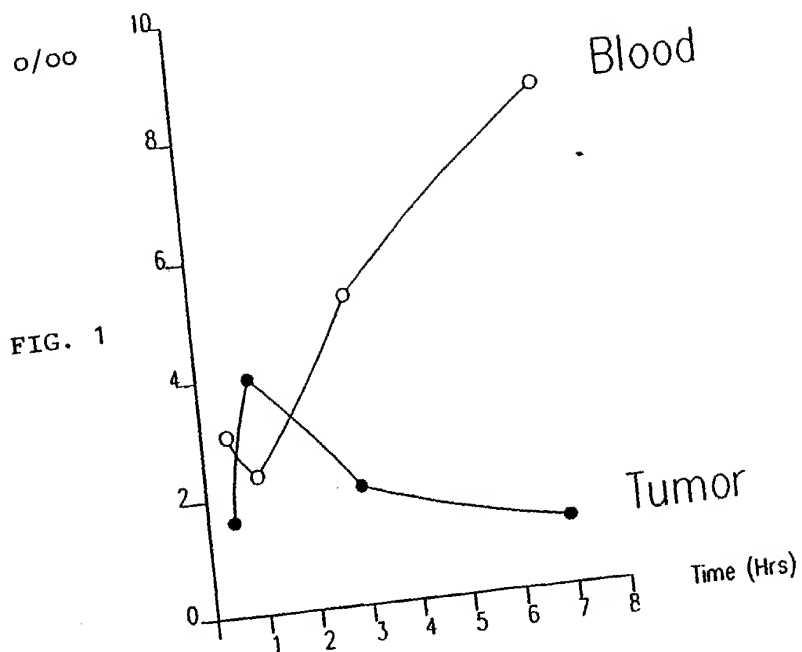
13. Process according to claim 9, wherein the protecting substance is starch.

35

13. Process according to claim 9 wherein the solvent used for extraction of the LDL is heptane.

14. Use of a composition according to claim 1 or a composition prepared according to claim 7 for therapeutic or diagnostic treatment of humans and non-human animals.

1/1





## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 93/00149

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 9/127, A61K 49/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, WPIL, CLAIMS, MEDLINE, EMBASE, CHEMICAL ABSTRACTS, CANCERLIT.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages      | Relevant to claim No. |
|-----------|---|-----------------------|
| A         | WO, A1, 8607540 (ONCHOLAB AB), 31 December 1986<br>(31.12.86)<br><br>-----<br><br>----- | 1-13                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 July 1993

Date of mailing of the international search report

27 -07- 1993

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Anneli Jönsson  
Telephone No. +46 8 782 25 00

# INTERNATIONAL SEARCH REPORT

In ternational application No.

PCT/FI 93/00149

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 14  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods (see PCT Rule 39.1(iv).
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

### Information on patent family members

PCT/FI 93/00149

Form PCT/ISA/210 (patent family annex) (July 1992)